Spring 5-2-2021

Determination of Metals and Microplastics in Sediment from Oyster Reefs in the Mississippi Sound

Shelby Goza

Determination of Metals and Microplastics in Sediment from Oyster Reefs in the Mississippi Sound

By
Shelby Goza

A thesis submitted to the faculty of the University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College

Oxford
May 2021

Approved by

Advisor: Dr. James Cizdziel

Reader: Dr. Nathan Hammer

Reader: Dr. Jason Ritchie

ACKNOWLEDGEMENTS

Firstly, I would like to thank my family and friends for their continual support throughout my undergraduate career. I would also like to express my deepest appreciation to my thesis advisor, Dr. James V. Cizdziel, as his guidance and assistance with regard to my undergraduate research activities has been invaluable to my success at the University of Mississippi, not to mention this project. I would also like extend thanks to my fellow research group members, with special appreciation for the guidance provided by Byunggwon Jeon and Kendall Wontor, who generously afforded me many hours of their time in my quest to complete this project, as well as Austin Scircle, who made this project possible by actually collecting the samples under study.

I would also like to thank my second and third readers, Dr. Nathan I. Hammer and Dr. Jason E. Ritchie, not only for their participation in the thesis process, but also for the help that each has extended to me during my time at the University of Mississippi. Lastly, I extend my gratitude to the Sally McDonnel Barksdale Honors College, and more broadly, the University of Mississippi community as a whole. The faculty and staff behind these institutions have made the rich and exciting undergraduate experience that I've enjoyed over the past few years possible. I'm beyond proud to be graduating from this university as an Honors student, and I will never stop being an Ole Miss Rebel.

ABSTRACT

This research centers on the determination of metals (Cd, Pb, V, Cr, Mn, Fe, Co, Ni, Cu) and microplastics (MPs) in marine sediment from oyster reefs in the Mississippi Sound Estuary. Oysters (*Crassostrea* virginica) are a vital part of the Gulf Coast economy, but their population has dramatically declined in recent years due to multiple stressors, including oil spills and fluctuations in salinity from flooding. Increasingly frequent flooding events also introduce high loads of MPs and sediments containing heavy metals from the Mississippi River. These pollutants can have deleterious effects on oyster biology; however, they have not been adequately studied at oyster reefs along the Gulf Coast. As filter-feeders, oysters are especially vulnerable to MPs and heavy metals. Moreover, MPs absorb certain contaminants, including heavy metals and hormone disruptors, exacerbating the situation by exposing the organisms to additional toxins.

Metals were leached from the sediment using microwave-assisted nitric acid digestion and determined using inductively coupled plasma mass spectrometry (ICP-MS). Concentrations ranged from 0.021 μg/g for Cd to 1024 μg/g for Fe. Concentration of the metals were compared between sites and with literature values. No metal concentrations exceeded the sediment quality guideline probable effect level, although Pb was close at a few sites, suggesting that heavy metal contamination at the site is not a major concern.

Putative MPs in the sediment were isolated using a $ZnCl_2$ separation followed by filtration and characterized using a stereomicroscope. Total MP counts in 20 g sediment samples ranged from 162 to 379. We observed between 22 and 31 fibers and fragments at sites 1 and 4, however, site 6 had 52 fibers and 56 fragments. Beads ranged from 6 to

319, with higher numbers in the 45-125 µm size fraction compared to the >125 µm size fraction.

Overall, this study is one of the first to assess both heavy metals and MPs at oyster reefs in the Mississippi Sound. While metal concentrations do not appear to be a risk based on current guidelines, MPs were prevalent in the sediment and may be resuspended during storm events, exposing oysters and other biota to the plastic pollution.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

Determination of Metals in Oyster Reef Sediment

1.1 Introduction

1.1.1 Sources and Implications of Heavy Metals in Marine Environments

Heavy metals such as Hg, Cd, and Pb stem from both natural and anthropogenic sources. High levels of these metals in the environment threaten the health of both humans and wildlife. The preservation of both freshwater and marine ecosystems has become an increasingly pressing issue in recent decades as the effects of multiple types of marine pollution become more evident and exceedingly difficult to remedy. Though multiple international agreements have been struck to mitigate these issues through regulations, many pollutants from both the present and past continue to disrupt aquatic ecosystems.

Heavy metal pollutants are persistent and a particularly difficult to clean up. Industrial activities such as mining and alloy processing release heavy metals that end up in rivers, lakes, and oceans (Clark, 1989). Agricultural byproducts like fertilizers and pesticides as well as sewage and urban runoff contain heavy metals, making this pollution an issue that extends well past the coastline (Melquiades & Appoloni, 2004). Heavy metals tend to become incorporated in marine sediment, conferring a continual risk to species in the vicinity (Clark, 1989). Heavy metals such as Hg are assimilated by phytoplankton and enter the food chain, increasing in concentration up the food web. Apart from the concern of marine species endangerment, health concerns extend to humans, as we consume seafood that may contain high levels of heavy metals.

Filter-feeders such as oysters and plants like seaweed are harvested by humans, processed, and eventually shipped to locations both near and far from the coastline. Much of the heavy metals and other chemicals accumulated by species like these remain in them until they are ingested by humans. Consumption of elevated levels of heavy metals pose a clear risk to human health.

1.1.2 Inductively Coupled Plasma – Mass Spectrometry

Inductively coupled plasma – mass spectrometry (ICP-MS) is an analytical technique used to quantify elements in samples. Following preparation, samples are typically nebulized within the sample introduction system, and the resulting aerosol is transferred to the argon plasma, which atomizes and ionizes the sample (Wilschefski & Baxter, 2019). The ions produced pass through the interface region and into the ion optics portion, which is made up of a set of many electrostatic lenses that focus the ions into a beam that passes into the quadrupole mass analyzer (Wilschefski & Baxter, 2019). The quadrupole separates the ions in the beam by their mass-charge ratio (m/z), and the final mass spectra is measured at the detector. Another type of mass analyzer, double-focusing, consists of magnetic and electric sectors. The combination allows high resolution capable of separating many isobaric interferences. We employed a sector field (double-focusing) instrument in this study. A simple diagram of the instrument is seen below (**Figure 1**).

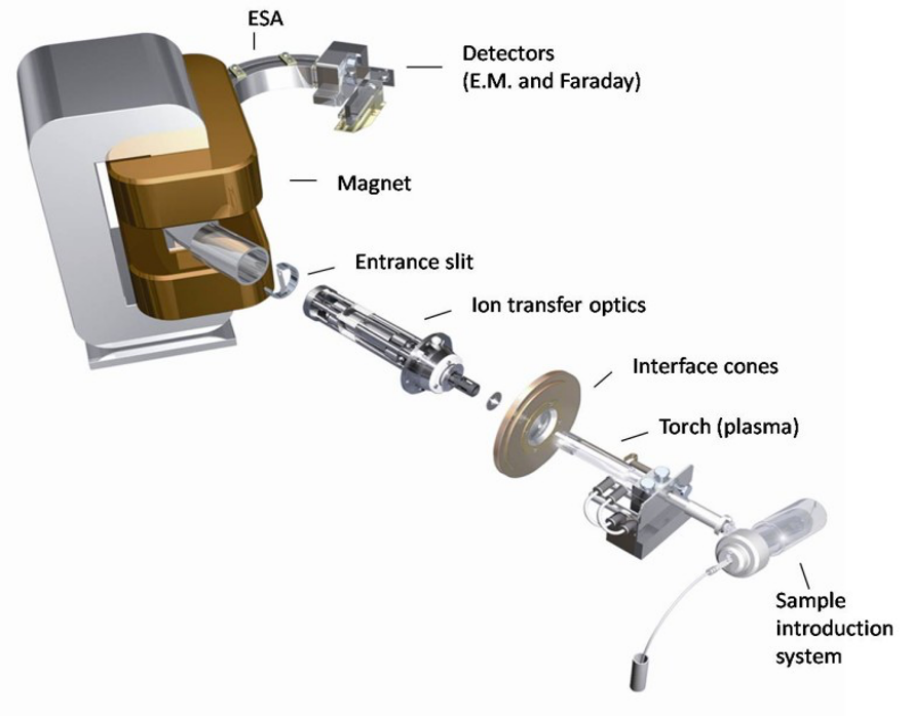

Figure 1: Simple Diagram of a sector field (double-focusing) ICP-MS (Courtesy of Thermo Electron Corporation).

The advantages of ICP-MS are its high sensitivity, wide elemental coverage, and simple sample preparation. Its ability to measure more than one element and their respective isotopes at a time makes it incredibly useful in trace element analysis in both biological and nonbiological samples (Nuttall et al., 1995). The drawbacks of ICP-MS lie in its high upfront and operational costs.

1.1.3 Prior Research

Several studies have been conducted using ICP-MS methodology in order to determine and chart the changing chemical makeup of marine ecosystems, shedding light on elevated levels of toxic metals in waterways that flow into larger marine environments as well as the oceans themselves (McComb et al., 2015; Kinuthia et al., 2020). Many metals included in these analyses occur naturally in marine environments and stem from

rock weathering and soil erosion. However, some naturally occurring metals are toxic and carcinogenic even at low concentrations. Moreover, metals that are relatively safe at low concentrations can become toxic when concentrations increase. Since industrialization, heavy metal concentrations have increased in aquatic ecosystems, especially those proximal to population, industrial, or agricultural centers (Jeon et al., 2020; Burton, 2002). Concentrations can reach toxic levels due to natural disasters and human contributions in the realms of mining, urban and agricultural runoff, industrial emissions, and nuclear reactions. Additionally, metal concentration increases resulting from natural and anthropogenic sources generally occur within a relatively short period of time (≤ 1 year) following emission on a global scale (Jeon et al., 2020).

As these contaminants enter waterways, such as the Mississippi River, they accumulate in the riverbed sediment or are carried into larger marine ecosystems such as estuaries or bays, where they similarly accumulate and persist, in part due to their reactivity with the sediment particles (Jeon, 2020). Organisms in marine environments are susceptible to heavy metal pollution. Sediments act as a reservoir of pollutants including heavy metals. Some metals in sediments can re-suspended or dissolve and be widely dispersed via currents (Burton, 2002). In the Mississippi Sound Cr and Ni have increased markedly in both sediment and shellfish since the late 1980s (Elston et al., 2005).

Though the potential for negative effects of metal accumulation in sediment are great in number, the standards by which we assess metal content in sediment are few, particularly in the U.S. (Burton, 2002). Sediment quality guidelines (SQGs) have been developed to assess toxicity by normalizing sediments based upon their organic carbon

concentration (Burton, 2002). Two of these SQGs, threshold effect level (TEL) and probable effects level (PEL), were utilized in this study. A TEL represents the concentration level below which effects rarely occur, and the PEL represents the concentration level above which effects are likely to occur (Burton, 2002). Additionally, comparison to the soil content of the global lithosphere provides valuable perspective on heavy metal levels in marine environments and their connection anthropological activities.

1.1.4 Purpose of this Study

The purpose of this study was to determine the heavy metal composition of marine sediment that makes up oyster reefs located in the Mississippi Sound portion of the Northern Gulf of Mexico near Bay St. Louis. Specific objectives were to determine the heavy metal concentration contained in sediment samples from six different oyster reef sites across the Mississippi Sound and further compare across sites, with literature values, and to sediment quality guidelines.

1.2 Materials and Methods

1.2.1 Study site

Marine sediment samples were collected from six sites within the Mississippi Sound portion of the Northern Gulf of Mexico, which are represented in **Figure 2** and **Table 1**. Three samples were collected from each site using an Ekman dredge.

Figure 2: Oyster Reef Sediment Collection Sites 1-6. Maps Data: Google, ©2021 INEGI.

Table 1: Sediment Collection Site Identifications and Locations		
Site	Site Name	Location Coordinates
Site 1	Non-reef Site Bay St. Louis	30.351007,-89.35467
Site 2	TNC Bay St. Louis	30.345059,-89.294855
Site 3	Mussel Watch BSL St. Stanislaus Reef	30.3023,-89.3272/ 30.30074,-89.319644
Site 4	Waveland Reef	30.272957,-89.370245
Site 5	Henderson Point Reef	30.292643,-89.271125
Site 6	Kittiwake Reef	30.332443,-89.165184

1.2.2 Analysis of sediment by ICP-MS

Three sediment samples per site were prepared via microwave acid digestion. 0.3 to 0.4 grams of sediment was weighed out for each sample and added to a Teflon microwave digestion vessel. Next, 3 mL of trace metal nitric acid (HNO_3) was added to each vessel, followed by 1 mL of trace metal hydrochloric acid (HCl). After each vessel had begun producing fumes, 6 mL of deionized water was added. Four blanks were likewise prepared with the same amounts of acid and water. The vessels were loaded into an Ethos Microwave from Milestone Inc. The samples were digested at 1200 W ramped up to 180° for 20 minutes and held at 180° for 30 more minutes, then allowed to cool before transfer to some centrifuge tubes and dilution to the mark with deionized water.

After an internal standard had been added, the solutions were diluted to 50 mL and subsequently subjected to vacuum filtration. After the filtration step had been performed, 5 mL of filtered solution for each sample was added to 15 mL tubes and diluted to 10 mL with deionized water.

Samples were analyzed using an Elemental XR ICP-MS from Thermo Fisher. Prior to analysis, the ICP-MS was tuned for sensitivity. Calibration was performed using a multielement standard (Spex Inc.) at the following concentrations: 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 ppb. Following calibration, triplicate samples from each of the six sites were tested. For accuracy, Standard Reference Material 1640a was included in the analyses to measure percentage recovery rates among trace metals tested.

1.3 Results and Discussion

1.3.1 Calibration, Quality Assurance, and Reference Material Recovery Data

Seven-point calibration curves were prepared for nine metals, including the heavy metals of Cd and Pb. Each of these metals had r^2 values >0.99 (**Table 2**).

Table 2: Calibration Curve Regression Equations and R-Squared Values

Metal	Equation	R squared
Cd	y = 0.0723x - 0.0003	1
Pb	y = 0.85x - 0.0112	0.9998
V	y = 0.76x - 0.0024	1
Cr	y = 0.8022x + 0.0216	0.9998
Mn	y = 1.0622x + 0.0132	1
Fe	y = 1.0005x + 0.7283	0.9985
Co	y = 0.9471x + 0.0018	1
Ni	y = 0.2551x + 0.0359	0.9997
Cu	y = 0.6003x + 0.0297	0.9996

To assess accuracy, NIST standard reference material 1640A was analyzed. Percentage recoveries for each of these metals are shown in **Table 3**. Only Co, Fe, and V had values outside the 75%-125% range (highlighted in yellow and red), so results for those elements in the samples should be viewed with caution.

Table 3: Percentage Recovery Values of Elements in SRM 1640a

Element	Conc. (ng/g)	Cert. Values (ng/g)	Rec. (%)
Cd	4.8	3.96	121
Pb	14.5	12	121
V	10.8	14.93	72
Cr	32.9	40.22	82
Mn	32.5	40.07	81
Fe	47.1	36.5	129
Co	10.7	20.08	53
Ni	20.0	25.12	80
Cu	64.6	85.07	76

1.3.2 Metal Concentration between Sites and Relative to Sediment Quality Criteria and Literature Values

Metal concentrations of samples (μg/g) for each site are given in **Table 4** and **Figures 3-6**. Of these concentrations, several displayed patterns across sites. A couple of noticeable trends were identified with regard to Site 6 (Kittiwake Reef). Site 6 exhibited Mn levels much higher than any other site tested, resulting roughly in a 4.8:1 ratio of Mn levels in site 6 compared to the average of sites 1-5. It had similarly higher levels of copper compared to other sites, displaying a ratio of ~2.3:1 of copper levels compared to the average of sites 1-5. This is shown in **Figure 3** and **Figure 4**.

Table 4: Selected Heavy Metal Concentrations (µg/g) and Standard Deviations in Oyster Reef Sites 1-6												
Metal	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6	
	Conc.	SD	Conc.	SD	Conc.	SD	Conc.	SD	Conc.	SD	Conc.	SD
Cd	0.762	±0.069	0.374	±0.016	0.663	±0.095	0.515	±0.039	0.867	±0.033	0.021	±0.005
Pb	70.4	±3.26	27.9	±0.92	75.4	±6.17	42.7	±2.34	84.0	±3.38	3.84	±1.54
V	2.91	±0.214	2.81	±0.284	3.37	±0.112	3.43	±0.205	3.33	±0.183	1.54	±0.257
Cr	1.77	±0.131	1.71	±0.137	1.89	±0.072	1.86	±0.144	1.89	±0.125	1.06	±0.253
Mn	10.7	±0.789	13.5	±1.82	23.4	±2.10	22.1	±1.15	24.8	±5.57	90.4	±30.6
Fe	1024	±71.0	865	±103	906	±85.9	945	±79.4	758	±48.4	442	±64.8
Co	0.475	±0.03	0.491	±0.049	0.448	±0.026	0.451	±0.023	0.403	±0.038	0.233	±0.018
Ni	0.59	±0.04	0.563	±0.075	0.691	±0.028	0.749	±0.054	0.699	±0.035	0.409	±0.076
Cu	0.228	±0.018	0.276	±0.059	0.233	±0.012	0.388	±0.054	0.249	±0.024	0.623	±0.419

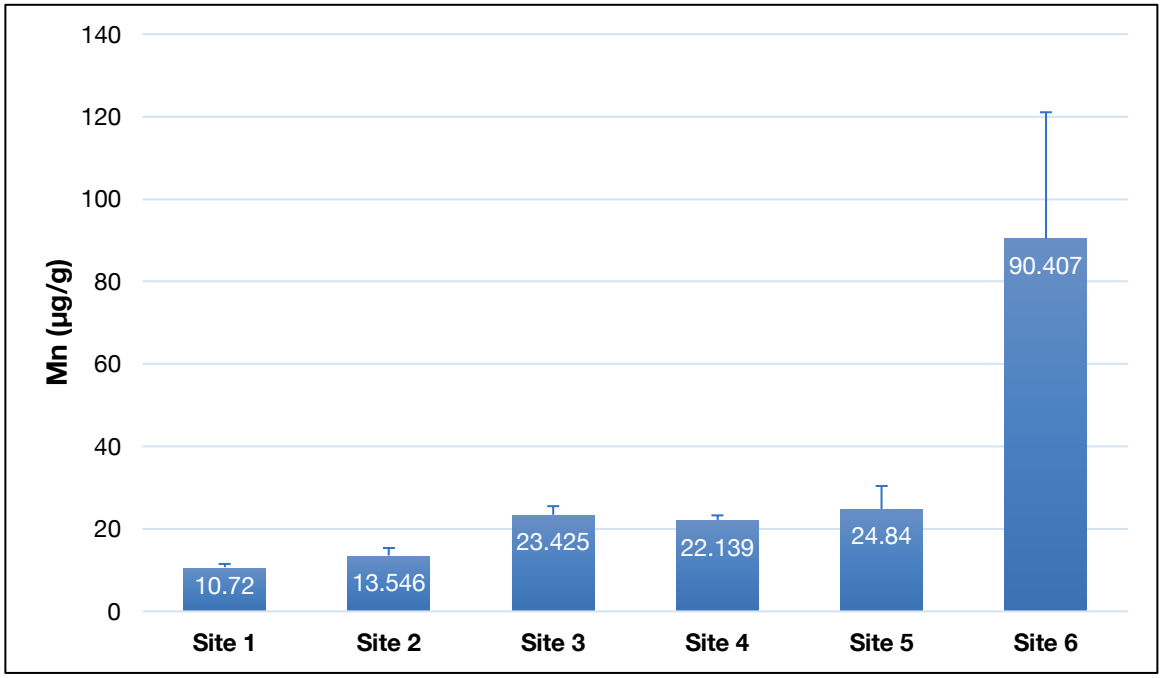

Figure 3: Concentration of Mn in Sediment from Oyster Reefs in the Mississippi Sound.

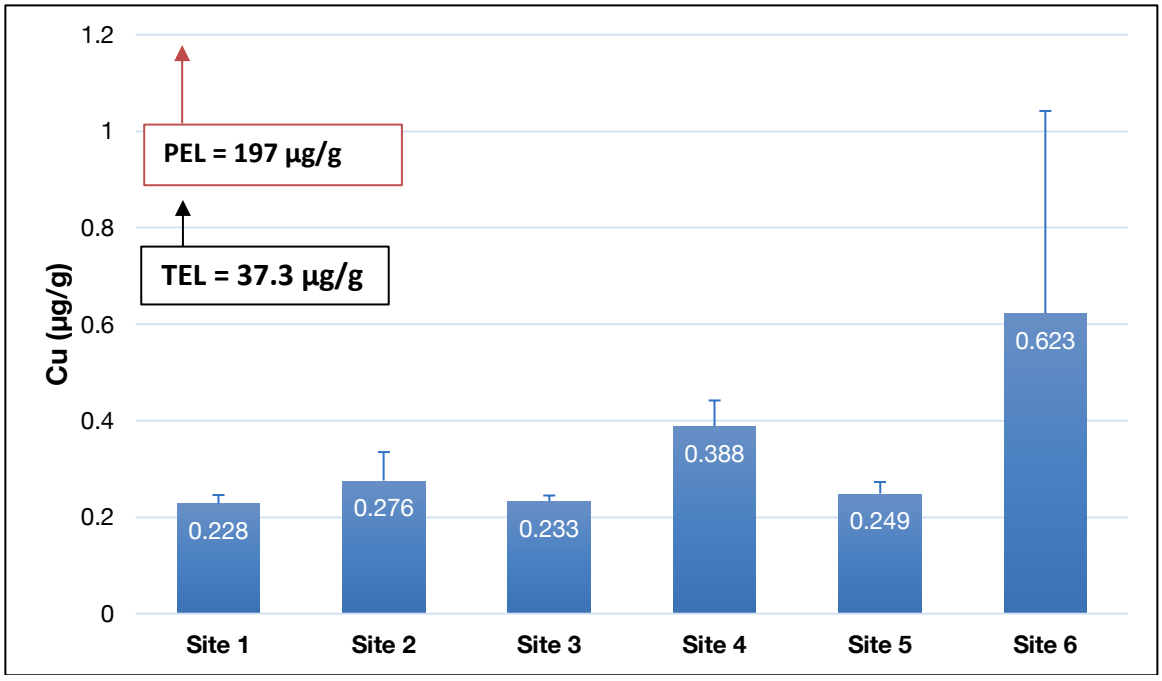

Figure 4: Concentration of Cu in Sediment from Oyster Reefs in the Mississippi Sound.

Considering the comparatively high levels of Mn seen in Site 6, it is possible that Mn levels are higher in Kittiwake Reef because it is located near wastewater or sewage

treatment effluent networks, which are considered major anthropogenic sources of environmental Mn (Howe et al., 2004). It is also the reef closest to Biloxi, which could also be contributing to elevated levels via gulf currents. Proximity to wastewater treatment networks could likewise explain the increased copper levels, as sewage usually contains copper as a result of corrosion of copper plumbing over time (Kiprop, 2021). Even so, the Cu concentration levels in each of the sites is well below the PEL as well as the TEL, meaning that toxic effects would rarely occur at these levels.

Beyond comparison of heavy metal concentrations between sites, these concentration values were also compared to concentrations in the global lithosphere and topsoil. Both Pb and Cd displayed values in some sites that were considerably higher than lithosphere averages. Pb concentrations in oyster reef sites 1-6 are compared to global lithosphere and topsoil values in **Figure 5**, and Cd concentrations of sites 1-6 are compared with their corresponding lithosphere and topsoil values in **Figure 6**.

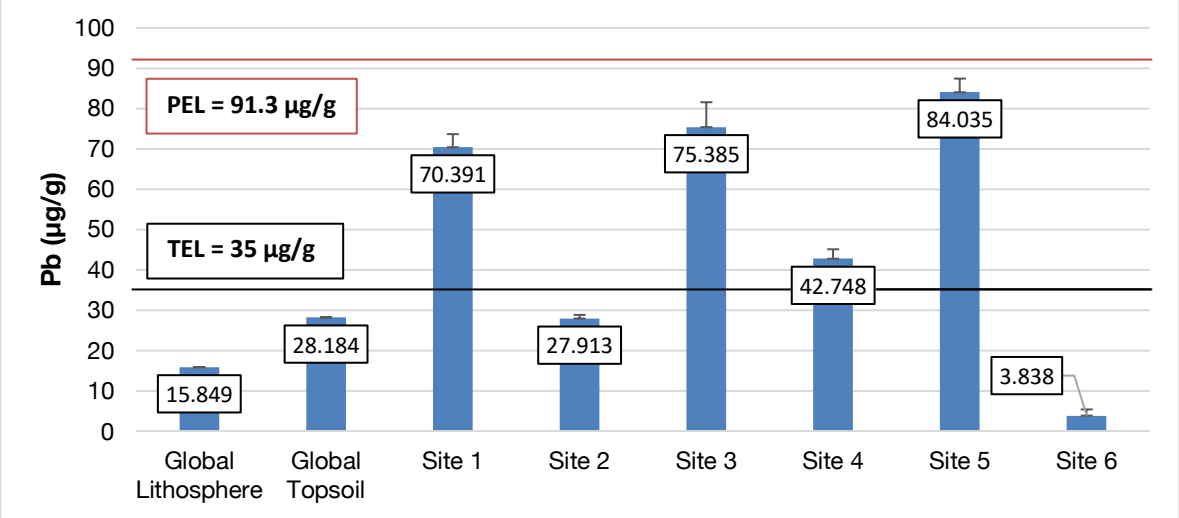

Figure 5: Pb concentration values obtained from oyster reef sites compared to global lithosphere and topsoil averages (Kabata-Pendias & Pendias, 2001).

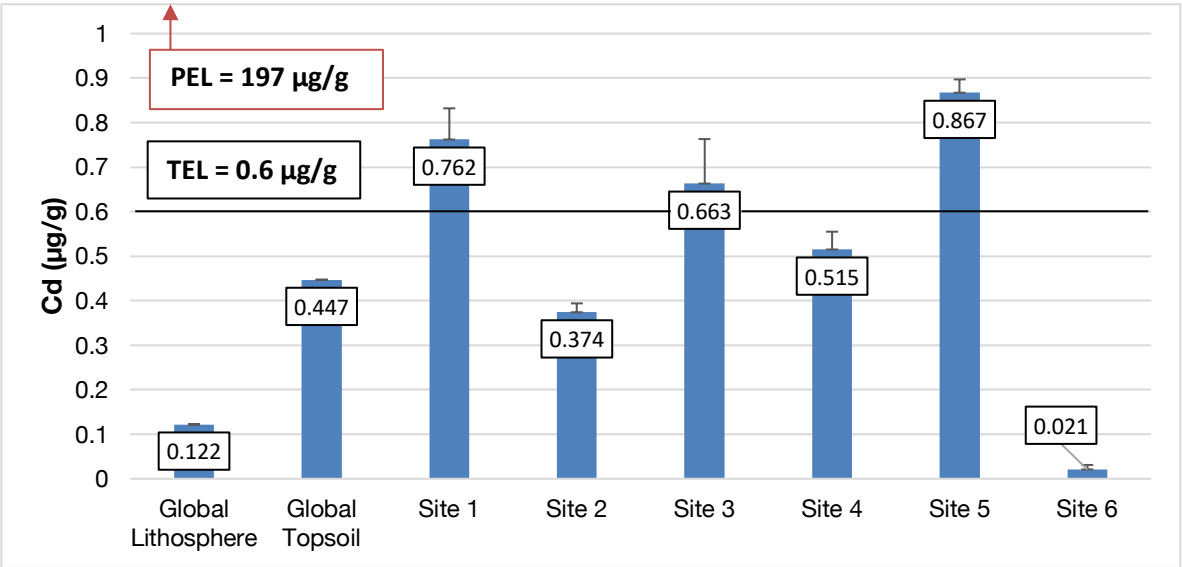

Figure 6: Cd concentration values obtained from oyster reef sites compared to global lithosphere and topsoil averages (Kabata-Pendias & Pendias, 2001).

As seen in the figures above, Pb concentration levels in all sites except Site 6 are higher than global lithosphere values. Of those sites, four of them display Pb concentration levels above that of global topsoil. This trend is mirrored in the case of Cd. Conversely, Pb and Cd differ in terms of TEL and PEL. The same four sites with Pb concentrations above the global topsoil averages (1, 3, 4, 5) also exceed the TEL, meaning that toxic effects only rarely occur in Site 2 and Site 6 with respect to Pb. No site exceeds the PEL threshold, meaning effects cannot be said to likely occur in any of the sites. However, Site 5 is only roughly 7 µg/g of Pb away from reaching that threshold, and Sites 1 and 3 are not far behind. With respect to Cd, three sites (1, 3, 5) exceed the TEL, meaning that toxic effects rarely occur in Sites 2 and 4. Even as Sites 1, 3, and 5 exceeded the TEL, their concentrations are sufficiently distant from the PEL, conferring a relatively low risk for toxic effects.

1.3.3 Possible Point Sources for Metal Pollution

Upon research into the anthropogenic processes that can contribute to Pb and Cd pollution, soap manufacturing was recognized as a factor. The pipeline cleaning associated with soap production processes has been determined as a major source of pollutants and provides a link to the heavy metal pollution resultant from soap industry effluents (Mustafa & Alradi, 2014). One such soap manufacturer, Pass Christian Soap Co., previously existed within the Bay St. Louis area until 2005 and is identified in **Figure 7**.

Figure 7: Pass Christian Soap Co. identified in the Bay St. Louis/MS Sound Area. Maps Data: Google, ©2021 INEGI.

Considering this soap manufacturer's proximity to oyster reef sites and their corresponding heightened levels of Pb and Cd compared to global lithosphere and topsoil values, it is logical that soap manufacturing in the Bay St. Louis area was possibly contributing to high concentrations of Pb and Cd in marine sediment nearby. Future work should determine the mechanisms and pathways by which land pollution reaches marine

sediment, potentially by air and water column sampling around coastal areas of the Mississippi Sound.

1.4 Conclusion

Concentrations of metals (Cd, Pb, V, Cr, Mn, Fe, Co, Ni, Cu) were determined in sediment at six oyster reefs in the Mississippi Sound. None of these metals exceeded their probable effect level at any site, although Pb, Mn, and Cd were close at certain sites and thus warrant additional monitoring. Relatively high levels of Mn in Site 6 may indicate proximity to municipal wastewater discharge and sewage sludge. Increased levels of Cd and Pb in Sites 1, 3, 4, and 5 imply anthropogenic pollution sources, which may include manufacturing in the Bay St. Louis area. Overall, we conclude that sediment metal concentrations confer a low risk to the oysters and the Mississippi Sound ecosystem as a whole, but that continued monitoring is recommended.

1.5 Future Work

In future studies, we recommend investigation of heavy metal concentrations in species living within the Mississippi Sound to gauge the level of bioaccumulation occurring within the larger food web, specifically with regard to species subject to commercial seafood industries, as they provide a direct link between marine life in these potentially toxic environments and direct human consumption. Future studies could also address the health outcomes of populations with high levels of seafood consumption in areas that sell seafood caught in the Mississippi Sound.

CHAPTER 2

Microplastic Pollution in Sediment from of Oyster Reefs in The Mississippi Sound

2.1 Introduction

2.1.1 Sources and Implications of Microplastics in Marine Environments

Despite the increased attention that the growing ubiquity of microplastics (MPs) in bodies of water and sediments has garnered in recent years, plastic production has been on an uptrend since the 1950s (Wright et al., 2013). Leading from the disposable one-use nature of many commercial plastics, much of it is disposed almost as quickly as it is produced, contributing to plastic accumulation in the environment at uncontrollable rates. Disposed plastic products eventually weather and degrade into smaller plastic particles referred to as MPs, which are defined as less than 5 mm in size. These particles are then subject to transport to coastal areas driven by wind and river systems (Wright et al., 2013). Degradation products of larger plastic debris are referred to as secondary MPs (Scircle, 2020). Additionally, some MPs released into the environment stem from microplastic beads intentionally manufactured at the sub 5 mm size. Referred to as primary MPs (Scircle, 2020), these plastics are generally produced for use in facial and other hygiene-based products (Smith, 2017).

Quantification of MPs has become an essential goal in environmentally driven research for several reasons. MP's ubiquity in marine environments has serious implications for marine health. MPs can accumulate in filter feeders such as oysters or mollusks, which not only causes adverse health effects, but also has implication for the health of animals feeding on them, some of which are humans (Smith, 2017).

Additionally, many of the MPs accumulating within food webs are subject to binding to other harmful chemicals present in the environment, meaning that MP accumulation is coupled with potential exposure to toxic pollutants (Smith, 2017). Each of these issues are compounded by the fact that MPs are relatively stable in the environment and are only growing in number and reach as plastic production continues (Smith, 2017).

2.1.2 Prior Research

Growing interest in MPs within the environmental chemistry community has been driven by an increasing number of studies (Barnes et al., 2010; Free et al., 2014; Li et al., 2017; Ng & Obbard, 2006) linking their presence to places both expected and unexpected. Population centers are significant sources of MP pollution, as they predictably generate large amounts of plastic waste. In terms of primary MPs, waste flowing from the cumulative and often daily use of personal care products across populations likely passes through wastewater treatment plants and into natural water systems (Tagg et al., 2015). Secondary MPs enter natural water systems around population centers slightly differently. Rather than being carried into waterways via wastewater discharge, many secondary MPs find their way into the environment by way of improper plastic waste disposal, i.e., littering (Li et al., 2017). Moreover, even if plastics are properly disposed of, they are still subject to degradation in landfills surrounding population centers, increasing the supply of MPs making their way into natural water systems through leachates and wind transport (Li et al., 2017). Even though MPs are usually found in higher concentrations around population centers, their reach also extends to the remote (Scircle et al., 2020). MPs have been identified in bodies of

water in tropical areas (Ng & Obbard, 2006), as well as lakes in isolated mountain areas (Free et al., 2014). The ubiquity of MPs in the environment cannot be understated, as their presence literally extends from pole to pole, polluting aquatic environments in the Arctic (Bergmann et al., 2016) and the Antarctic (Barnes et al., 2010). The pervasiveness of MPs extends to marine organisms, as they enter aquatic food webs though filter feeders. Once MPs accumulate in sediment, they become introduced to oysters once the sediment is disturbed, either by way of marine organism traffic generally or harvesting operations for commercial use.

2.1.3 Purpose of this Study

The purpose of this study was to quantify and characterize shapes and sizes of MPs in marine sediment samples from oyster reefs in the Mississippi Sound portion of the Northern Gulf of Mexico near Bay St. Louis. In completing MP quantification with respect to the oyster reef sites under study, the pollution profile of said sites is broadened beyond the scope of heavy metal composition. MP quantification coupled with heavy metal determination, which is outlined in Chapter 1, sees importance in the fact that MPs can sorb heavy metals and further introduce them into food webs (Smith, 2017). While most directly a risk for filter feeding species such as oysters, MPs, heavy metals, and their combinations further represent an already ubiquitous problem for environmental and human health that continues to intensify as plastic production and use continues.

2.2 Materials and Methods

2.2.1 Study site

Marine sediment samples were collected from six sites within the Mississippi Sound portion of the Northern Gulf of Mexico, (**Figure 3** and **Table 1**). Three samples were collected from each site using an Ekman dredge.

Figure 2: Oyster Reef Sediment Collection Sites 1-6. Maps Data: Google, ©2021 INEGI.

Table 1: Sediment Collection Site Identifications and Locations		
Site	Site Name	Location Coordinates
Site 1	Non-reef Site Bay St. Louis	30.351007,-89.35467
Site 2	TNC Bay St. Louis	30.345059,-89.294855
Site 3	Mussel Watch BSL Location/St. Stanislaus Reef	30.3023,-89.3272/ 30.30074,-89.319644
Site 4	Waveland Reef	30.272957,-89.370245
Site 5	Henderson Point Reef	30.292643,-89.271125
Site 6	Kittiwake Reef	30.332443,-89.165184

Of the six sites represented above, three were selected for MP quantification: Site 1, Site 4, and Site 6. With these sites, characterizations can be made in terms of MP levels both within and outside the Bay of St. Louis.

2.2.2 Density Separation, Filtering, and Optical Quantification of Microplastics

Two sediment samples per site were prepared via $ZnCl_2$ density separation and subsequent filtering. Approximately 20 g of sediment was weighed out from each site and added to separate beakers previously rinsed three times with Millipore water. Next, the beakers were filled to 100 mL with $ZnCl_2$ and covered with foil, which was followed by stirring for five minutes at <350 RPM to ensure minimal fracturing of MPs contained in samples. The solutions were then allowed to settle for twenty-four hours.

Following the waiting period, the top fraction of each $ZnCl_2$ solution was extracted using a pipette and transferred to separate beakers, which were subsequently covered in foil. The original $ZnCl_2$ solutions were then filled back to 100 mL with $ZnCl_2$, covered with foil, and stirred again at >350 RPM followed by another twenty-four-hour waiting period. This process was repeated once more, followed by a third and final top fraction extraction of the $ZnCl_2$ solution into the separate beakers. Three extractions were performed for each sample because previous research (Corradini et al., 2019) indicated that three extractions are sufficient to achieve high recovery rates.

After a total of three extractions had been performed, extraction solutions containing MPs in $ZnCl_2$ were separated into two aliquots by pouring half of each solution into a separate beaker after stirring with a glass stir rod, creating duplicate samples to subject to filtering. Samples were then poured into a sieve apparatus characterized by an upper >125 µm portion and a lower >45 µm portion. This process was repeated for each sample, as well as two $ZnCl_2$ blanks. Once $ZnCl_2$ had been collected in the bottom of the apparatus, each filter was washed separately into beakers

with Millipore water. The resulting water/MP mixtures were then subjected to vacuum filtration using Whatman 47 mm 0.4 μm pore size filters, creating a total of fifteen samples. There would have been sixteen, however, the Site 1A sample was poured directly onto the >45 μm sieve instead of the >125 μm sieve, resulting in only one size fraction for Site 1A. The resultant filters were then placed in labeled sample holders and analyzed using a stereomicroscope.

2.3 Results and Discussion

2.3.1 Sample Microplastic Quantification and Characterization

Total combined MP counts of duplicate samples and averages in terms of number of MPs per 10 g sample of Sites 1, 4, and 6 are outlined below in **Table 5** and **Figure 8**. It is important to note that MP counts are putative, as the individual MPs themselves were not analyzed in terms of chemical properties.

Table 5: Amounts and Morphologies of Microplastics in Sediment from Oyster Reefs in the Mississippi Sound

Shape	Fiber	Fragment	Bead	Total
Site 1A >45 µm	2	4	0	6
Site 1B >125 µm	2	1	1	4
Site 1B >45 µm	22	17	113	152
Site 1 Total	26	22	114	162
Site 1 Average	13	11	57	81
Site 4A >125 µm	2	4	1	7
Site 4A >45 µm	10	3	17	30
Site 4B >125 µm	1	13	31	45
Site 4B >45 µm	16	11	270	297
Site 4 Total	29	31	319	379
Site 4 Average	15	16	160	190
Site 6A >125 µm	0	4	1	5
Site 6A >45 µm	10	33	0	43
Site 6B >125 µm	31	3	0	34
Site 6B >45 µm	11	16	5	32
Site 6 Total	52	56	6	114
Site 6 Average	26	28	3	57

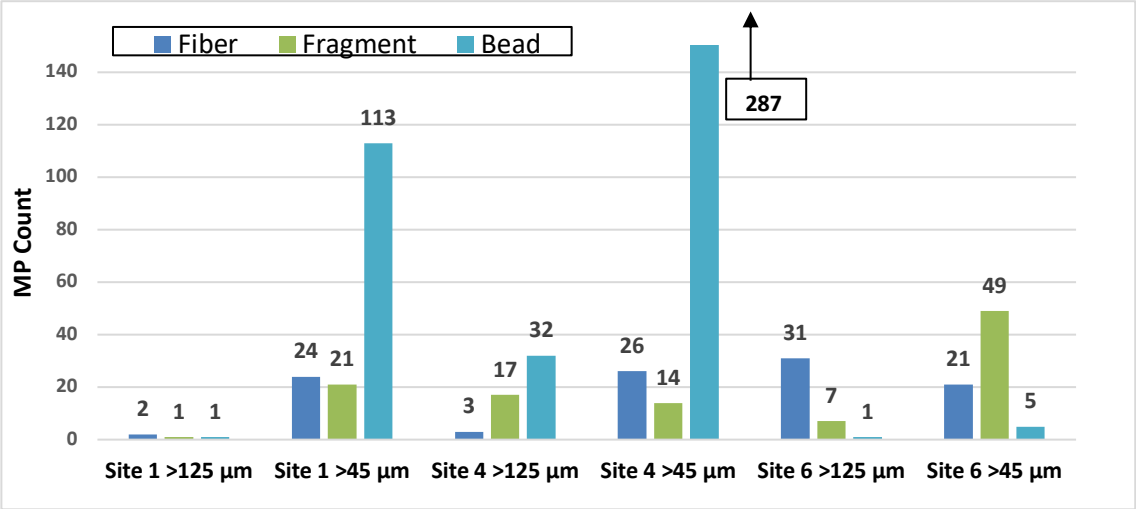

Figure 8: Combined Counts and Shapes of MPs in Oyster Reef Sites 1, 4, and 6.

As seen above, Site 1 had not only the least number of MP fibers, but also MP fragments. Additionally, Site 6 displayed the lowest total number of MP beads compared to Sites 1 and 4. However, Site 6 also contained the highest numbers of both fibers and fragments. A fragment and a fiber from Site 6 can be seen in **Figure 9** and **Figure 10**, respectively.

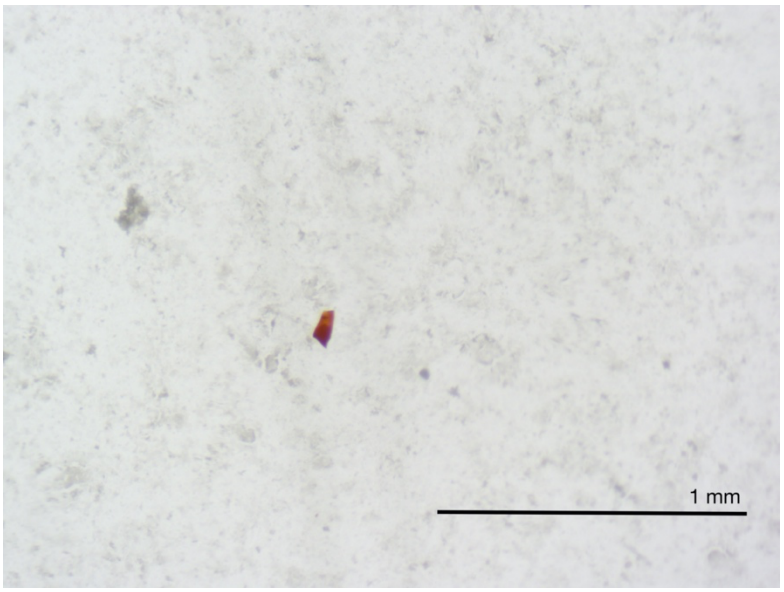

Figure 9: MP fragment identified in >45 µm size fraction of Site 6A sample using a stereomicroscope in conjunction with AmLite imaging software.

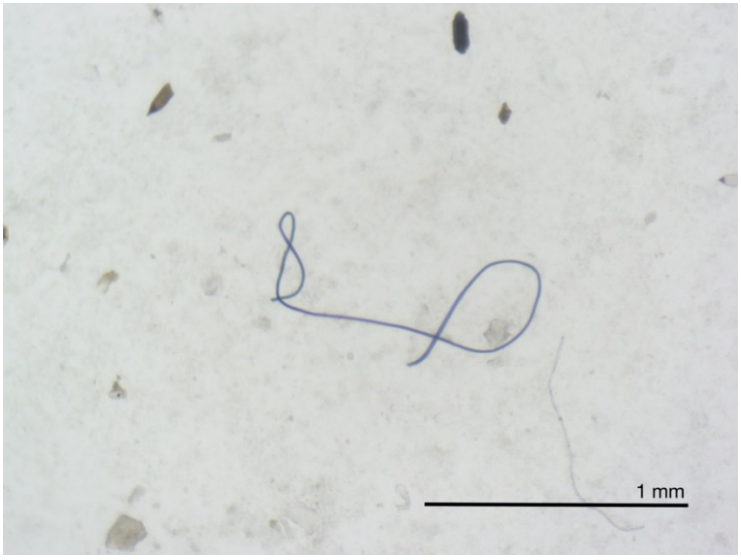

Figure 10: MP fiber identified in >45 µm size fraction of Site 6B sample using a stereomicroscope in conjunction with AmLite imaging software.

Site 4 contained the highest levels, by far, of MP beads. In the >45 µm size fraction alone, the number of MP beads is more than double that of the total bead counts of the other sites combined. A group of MP beads observed in a Site 4 sample is illustrated below in **Figure 11**.

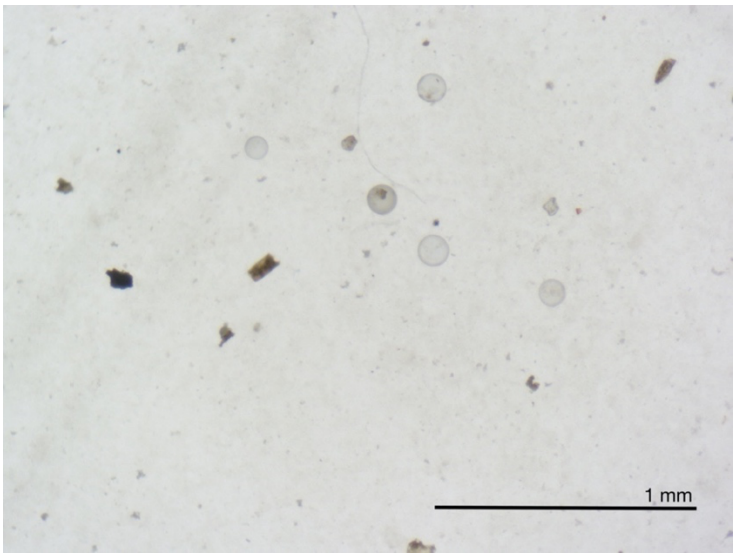

Figure 11: MP beads identified in >45 µm size fraction of Site 4B sample using a stereomicroscope in conjunction with AmLite imaging software.

High MP bead numbers in Site 4 samples are likely due to its comparably closer proximity to channels carrying waste accumulated by Mississippi River flood waters that have made their way to the Gulf of Mexico. Said flood waters are introduced into Lake Pontchartrain via the Bonnet Carre Spillway, located west of Site 4. This reasoning can be extended to the low numbers of MP beads seen in Site 6, which is the site farthest away from the Bonnet Carre Spillway. However, Site 6 still saw the highest numbers of both fibers and fragments. This can likely be attributed to its proximity Gulfport and Biloxi, MS, two major population centers on the coast east of Site 6. Though Site 1 observed the lowest total MP counts of fibers and fragments, it still had appreciable numbers of MP beads. As Site 1 is located in the Bay of St. Louis, it is probable that MP counts can be attributed to proximity to both the Bay St. Louis area as well as a variety of channels flowing into the bay.

With regard to all sites under study, higher numbers of MP beads were seen in the >45 μm size fraction than the >125 μm size faction. This trend is representative of an MP trend broader than beads specifically. As seen in **Figure 11**, many MP beads (and certainly fragments) contained in samples within this study are significantly smaller than the 5 mm cutoff for MP classification. As some of these plastics are further degraded by environmental and anthropogenic forces, they are approaching the possibility of being small enough to pass through biological membranes, which carries a significant risk for the health of all marine life and certainly human life (Li et al., 2018).

2.4 Conclusion

Putative MPs in sediment from several oyster reef sites within the Mississippi Sound were quantified and their morphology assessed. Numbers of MP fibers, fragments, and beads were determined using duplicate samples collected from each of three oyster reef sites. Site 6 displayed the highest levels of MP fibers and fragments, whereas Site 4 contained the highest number of MP beads. Site 1 was lowest in both fiber and fragment totals. In terms of average MP counts per 10 g sample, Sites 1, 4, and 6 contained 81, 190, and 57, respectively. High numbers of fibers and fragments in Site 6 could be attributed to the site's proximity to a major population center, Gulfport, MS. Elevated numbers of MP beads in Site 4 could likewise be attributed to this site's close proximity to the waste carried by Mississippi River flood waters. Despite low fragment and fiber counts in Site 1, its MP count average was still substantially higher than that of Site 6, meaning that it is likely more contaminated with MPs generally. Even so, Site 4 still dwarfs Site 1 with regard to MP average count, conferring MP pollution levels more than twice that of Site 1 and more than three times the average of Site 6. These wide differentials, though they have been demonstrated in sediment, have yet to be explored in terms of MP prevalence in the oysters themselves and the food webs that they exist within. Evaluating MPs in oyster populations, specifically those harvested for human consumption, could represent an important point of future research with far reaching implications for the oysters themselves, those that consume them, and the economic futures in the Mississippi Sound that they influence. Considering these conclusions and the nature of MP pollution broadly, levels of MPs assessed within this study are indicative of the present results of anthropogenic processes occurring not only near the

Northern Gulf of Mexico, but also proximal to the Mississippi River largely and those waterways that empty into it. As manufactured plastic products continue to be a constant in everyday life, their ubiquity will be mirrored, if not expanded upon, in the spread of their degradation products.

REFERENCES

Adams, C., Brand, C., Dentith, M., Fiorentini, M., Caruso, S., & Mehta, M. (2020). The use of pXRF for light element geochemical analysis: A review of hardware design limitations and an empirical investigation of air, vacuum, helium flush and detector window technologies. *Geochemistry: Exploration, Environment, Analysis, 20*(3), 366-380. doi:10.1144/geochem2019-076

Barnes, D. K., Walters, A., & Gonçalves, L. (2010). Macroplastics at sea around Antarctica. *Marine environmental research*, *70*(2), 250-252.

Bergmann, M., Sandhop, N., Schewe, I., & D'Hert, D. (2016). Observations of floating anthropogenic litter in the Barents Sea and Fram Strait, Arctic. *Polar biology*, *39*(3), 553-560

Burton Jr, G. A. (2002). Sediment quality criteria in use around the world. *Limnology*, *3*(2), 65-76.

Clark, R. B., Frid, C., & Attrill, M. (1989). *Marine pollution* (Vol. 4). Oxford: Clarendon Press.

Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E., & Geissen, V. (2019). Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. *Science of the total environment*, *671*, 411-420.

Elston, R., CAKE, E. W., Humphrey, K., Isphording, W. C., & RENSEL, J. J. (2005). Dioxin and heavy-metal contamination of shellfish and sediments in St. Louis

Bay, Mississippi and adjacent marine waters. *Journal of Shellfish Research*, *24*(1), 227-241.

Free, C. M., Jensen, O. P., Mason, S. A., Eriksen, M., Williamson, N. J., & Boldgiv, B. (2014). High-levels of microplastic pollution in a large, remote, mountain lake. Marine pollution bulletin, 85(1), 156-163.

Greany, K.M. (2005) An Assessment of Heavy Metal Contamination in the Marine Sediments of Las Perlas Archipelago, Gulf of Panama. Master of Science Thesis, Heriot-Watt University, Edinburgh.

Howard, J. (2019, August 02). Marine pollution, explained (1145203944 861680218 R. Stanton, Ed.). Retrieved January 05, 2021, from https://www.nationalgeographic.com/environment/oceans/critical-issues-marine-pollution/

Howe, P., Malcom, H., & Dobson, S. (2004). *Manganese and its Compounds: Environmental Aspects* (pp. 1-70, Rep. No. 63). Geneva, Switzerland: World Health Organization.

Jeon, B., Scircle, A., Cizdziel, J. V., Chen, J., Black, O., Wallace, D. J., ... & Hurley, J. P. (2020). Historical deposition of trace metals in a marine sapropel from Mangrove Lake, Bermuda with emphasis on mercury, Pb, and their isotopic composition. *Journal of Soils and Sediments*, *20*(4), 2266-2276.

Kabata-Pendias, A., & Pendias, H. (2001). *Trace elements in soils and plants*. Boca Raton, FL: CRC Press.

Kiprop, J. (2021, January 5). What Are The Sources And Effects Of Copper Pollution In The Environment? Retrieved February 19, 2021, from https://www.worldatlas.com/articles/what-are-the-sources-and-effects-of-copper-pollution-in-the-environment.html

Li, J., Liu, H., & Chen, J. P. (2018). Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. *Water research*, *137*, 362-374.

McComb, J. Q., Han, F. X., Rogers, C., Thomas, C., Arslan, Z., Ardeshir, A., & Tchounwou, P. B. (2015). Trace elements and heavy metals in the Grand Bay National Estuarine Reserve in the northern Gulf of Mexico. *Marine pollution bulletin*, *99*(1-2), 61–69. https://doi.org/10.1016/j.marpolbul.2015.07.062

Melquiades, F. L., & Appoloni, C. R. (2004). Application of XRF and field portable XRF for environmental analysis. *Journal of Radioanalytical and Nuclear Chemistry, 262*(2), 533-541. doi:10.1023/b:jrnc.0000046792.52385.b2

Mustafa, Nazik & Alradi, Gamal. (2014). Environment Health Impacts of Soap Industry Effluents: Case Study of Soap Collectors at Alfatah District, Omdurman Sudan.

Ng, K. L., & Obbard, J. P. (2006). Prevalence of microplastics in Singapore's coastal marine environment. *Marine pollution bulletin*, *52*(7), 761-767.

Nuttall, K. L., Gordon, W. H., & Ash, K. O. (1995). Inductively coupled plasma mass spectrometry for trace element analysis in the clinical laboratory. *Annals of clinical and laboratory science*, *25*(3), 264–271.

Sapkota, Y., McDonald, L. M., Griggs, T. C., Basden, T. J., & Drake, B. L. (2019). Portable X-Ray Fluorescence Spectroscopy for Rapid and Cost-Effective Determination of Elemental Composition of Ground Forage. *Frontiers in plant science*, *10*, 317. https://doi.org/10.3389/fpls.2019.00317

Scircle, A. (2020). *Microplastic Pollution in the Mississippi River and the Mississippi Sound Estuary: Concentrations, Sizes, Types, Loadings, and MethodDevelopment* (Doctoral dissertation, The University of Mississippi).

Scircle, A., Cizdziel, J. V., Missling, K., Li, L., & Vianello, A. (2020). Single-Pot Method for the Collection and Preparation of Natural Water for Microplastic Analyses: Microplastics in the Mississippi River System during and after Historic Flooding. *Environmental toxicology and chemistry*, *39*(5), 986-995.

Smith, A. (2017). Micro Plastics and Their Implications for Human Health: An Environmental Justice Approach. *ENV 334 Environmental Justice*. 3. https://digitalcommons.salve.edu/env334_justice/3

Soares de Almeida, G., Marques, E.D., José da Silva, F., Pinto, C.P., Silva- Filho, E.V., 2019. Application of pXRF (Field Portable X-ray Fluorescence) Technique in fluvial sediments geochemical analysis – Bule Stream, Minas Gerais State, Brazil. Journal of Sedimentary Environments, 4 (2): 143-158.

Tagg, Alexander S., Melanie Sapp, Jesse P. Harrison, and Jesús J. Ojeda. "Identification and quantification of microplastics in wastewater using focal plane array-based reflectance micro-FT-IR imaging." *Analytical chemistry* 87, no. 12 (2015): 6032-6040.

Turner, Andrew & Taylor, Alex. (2018). On site determination of trace metals in estuarine sediments by field-portable-XRF. Talanta. 190. 10.1016/j.talanta.2018.08.024.

Tykot R. H. (2016). Using Nondestructive Portable X-ray Fluorescence Spectrometers on Stone, Ceramics, Metals, and Other Materials in Museums: Advantages and Limitations. *Applied spectroscopy*, *70*(1), 42–56. https://doi.org/10.1177/0003702815616745

Wilschefski, S. C., & Baxter, M. R. (2019). Inductively Coupled Plasma Mass Spectrometry: Introduction to Analytical Aspects. *The Clinical biochemist. Reviews*, *40*(3), 115–133. https://doi.org/10.33176/AACB-19-00024

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: a review. *Environmental pollution*, *178*, 483-492.

Yang, L., Mester, Z., Abranko, L., & Sturgeon, R. E. (2004). Determination of total Chromium in seawater BY Isotope Dilution Sector Field ICPMS Using GC Sample INTRODUCTION. *Analytical Chemistry, 76*(13), 3510-3516. doi:10.1021/ac0498664